

LSB speaker discuss the role of RNase H2 in Aicardi- Goutieres Syndrome

By Deepa Singh

A seminar at NIEHS June 21 focused on two interesting consequences of unremoved ribonucleotides misincorporated into DNA - genomic instability and growth arrest.

[Andrew Jackson, Ph.D.](#),

(<http://www.hgu.mrc.ac.uk/people/a.jackson.html>)

a clinical geneticist in the Medical Research Council Human Genetics Unit at the University of Edinburgh, provided clear evidence that misincorporated ribonucleotides are the most common endogenous base lesions in the mammalian genome, occurring at a frequency of least 1 million sites per replicating cell.

The talk, hosted by Laboratory of Structural Biology (LSB) visiting fellow Anders Clausen, Ph.D., also established that the ribonuclease H2 (RNase H2) enzyme is responsible for removing these misincorporated ribonucleotides, and that mutations in this enzyme cause Aicardi-Goutieres syndrome (AGS) in humans.

AGS is an autosomal-recessive disorder caused by mutations in the genes encoding all three subunits of a heterotrimeric RNase H2, (H2A, H2B, and H2C). According to Jackson, AGS mimics viral infection in several significant ways and has similarities to autoimmune disorders, suggesting that a better understanding of the cause and progression of AGS may also yield insights into disease processes involved in other conditions (see [text box](#)).

RNase H2 in mammals - growth defects and viability

During his graduate studies, Jackson focused on the genes whose dysfunction can lead to neurological disorders, and was also part of the group that mapped the two AGS gene loci AGS1 and AGS2 using homozygosity mapping. Later, Jackson's group, along with Yanick Crow, Ph.D., of the University of Manchester, were involved in the identification of three different genes, which, when mutated, can cause AGS. Research in his lab has also shown the nature of human RNase H2 protein complex, for the first time, using molecular genetics.

Through some of his protein structural work, Jackson demonstrated that AGS-causing mutations in RNase H2 were clustered around the catalytic site of the enzyme, or situated at the interface of the three subunits. Most of these latter mutations destabilized the protein, due to their reduced level of expression in the cell, and, therefore, had reduced level of enzyme function.

To get insight into the biological role of RNase H2 *in vivo*, Jackson's group has created an RNase H2 mouse model. "The aim was to generate a neurological model, which would more accurately reflect the human condition, AGS," explained Jackson. However, a knock-in mouse model containing an A174T mutation in exon 7 of the H2B subunit, which recapitulates the most common human mutation, survived without any convincing phenotypes. But *Rnaseh2bE2o2X/E2o2X* mice, in which a stop codon was introduced serendipitously, was required for embryonic viability of mice, as the homozygous animals with the same alleles were not viable. These mice were also reduced in size from gastrulation, and this growth arrest was due to the activation of p53, which could be the consequence of activated DNA damage signaling, such as single or double-strand breaks in DNA.

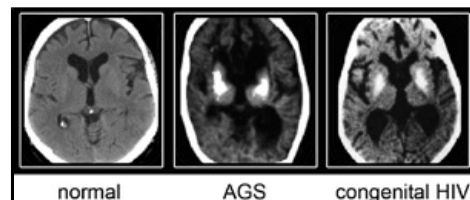
RNase H2, a surveillance enzyme

Jackson emphasized that in mammals, RNase H2 DNA repair activity is required for the correct removal of misincorporated ribonucleotides embedded within the genomic DNA. In RNase H2 null cells, either single or di-ribonucleotides are covalently incorporated by major replicative polymerases during DNA replication. According to Jackson, "Ribonucleotides are the most common noncanonical nucleotides found in mammalian DNA." Much of this work was inspired by similar studies reported on ribonucleotide misincorporation into DNA by yeast DNA replicative polymerases, from the NIEHS LSB DNA Replication Fidelity Group, headed by [Thomas Kunkel, Ph.D.](#)

Jackson concluded his talk by describing the good (presence of RNase H2), the bad (no RNase H2), and the ugly (reduced levels of RNase H2) consequences of ribonucleotides in the genome. Ribonucleotides can act as a signal for the mismatch repair



Clausen, left, and Jackson relaxed, following a talk that attracted a standing-room-only audience of scientists from NIEHS and area biomedical research centers. (Photo courtesy of Steve McCaw)



When he discussed these images of the brain, Jackson said he was struck by the similarities between the AGS brain and the brain of an individual infected at birth with the human immunodeficiency virus (HIV). (Photo courtesy of Andrew Jackson)

proteins, when errors are introduced during leading strand DNA synthesis, which is evident from work by [Scott Lujan, Ph.D.](#), an Intramural Research Training Award fellow in Kunkel's group. While reduced RNase H2 activity could be tolerated by the cell, potentially causing auto-inflammation as seen in AGS, lack of RNase H2 activity can cause genome instability.

(Deepa Singh, Ph.D., is a visiting fellow in the NIEHS Mechanisms of Mutation Group.)

Nucleases that mimic viral infection

AGS is a syndrome that mainly affects the brain, the immune system, and the skin. The clinical feature of AGS, a severe autoimmune disorder, mimics those of acquired *in utero* viral infection of the brain. This syndrome mostly affects newborns and infants, resulting in severe mental and physical handicaps.

Many newborns with AGS do not show any signs of the disorder at birth, but later on develop feeding problems, become irritable, develop fever, start having seizures, have abnormal posturing of limbs, and then progress to a state of profound neurological disability. During this progression, white blood cells and inflammation-associated molecules can be detected in the cerebrospinal fluid that surrounds the brain and spinal cord. These abnormal findings are consistent with inflammation and tissue damage in the central nervous system.

The symptoms, such as chilblain skin lesions on fingers, toes, and ears that manifest in 40 percent of the patients, are remindful of the autoimmune disease Systemic Lupus Erythematosus with cutaneous involvement. Most people with this disorder do not survive past childhood, but some affected individuals, with later onset and milder neurological problems, may live into adulthood. Loss of function mutations in the genes of TREX1, SAMHD1, and RNase H2 has been identified in patients with AGS.

Because AGS is a monogenic disorder with a defined molecular basis, Jackson's group is using it as a model for common autoimmune disease, to explore the cellular pathogenesis and molecular pathways implicated in nucleic acid-triggered inflammatory responses.

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